PERIODS OF SUSCEPTIBILITY IN THE DIFFERENTIATION OF UNFERTILIZED EGGS OF AMPHITRITE.

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While studying the unfertilized egg of *Amphitrite* at the Marine Biological Laboratory, Wood's Holl, Mass., I verified Fischer's [1] result that the eggs could be caused to develop cilia by squirting them from a pipette, by transferring them from one dish to another, or by some other sort of mechanical agitation. I believe however that it is inadmissible to speak of this development as parthenogenesis, meaning the production of a normal embryo from an unfertilized egg. Ciliated, swimming structures result, but their differentiation takes place with only partial or abnormal and usually without any definite segmentation. I will discuss the morphology of these processes in another paper. Lillie [2] has shown clearly a similar differentiation in the *Chætopterus* egg.

In addition to Fischer's results, I found: (1) At least two critical periods in which the egg is highly susceptible to mechanical stimulation, one period thirty to forty-five minutes, the other eighty to one hundred minutes after they are removed from the body and placed in sea-water; (2) slight agitation is more effective in the second period than in the first; rougher handling is better in the first than in the second in which the eggs are more easily broken into fragments. (3) Frequent and moderate squirting after thirty to fifty minutes seems more effective than one hard squirting after the same time.

In my early experiments with certain salt solutions, the results were sometimes discrepant, and there was great variableness in the number of swimming eggs obtained under apparently identical conditions. About this time Fischer's paper came into my hands. He had shown that "parthenogenetic development can be produced by adding a small amount of Ca-salt to sea-water"

[1] Fischer, Martin H., *Am. Jour. Phys.*, 1902, III., p. 301.

[2] Lillie, F. R., *Archiv für Entwickelungsmechanik der Organismen*, 1902, XIV., p. 377.

and by "mechanical agitation." "The unfertilized eggs of *Amphitrite*," he says, "develop to the trochophore stage if, after residence in sea-water from one half to one hour, they be squirted from a small nozzled pipette into another dish of sea-water." "The method is an uncertain one," depending upon "state of ripeness," and a "previous residence in sea-water or in one of the sea-water-salt solution mixtures is essential." He had noticed that some eggs are very sensitive to "mechanical manipulation," but rarely develop when treated "immediately after they are cut out of the body of the animal."

Already convinced that there was a time-factor to be considered, I planned the following series of experiments. In each series a set of eggs, removed from a single female at the same time, was used. Due precautions were taken to prevent fertilization by previously washing the female thoroughly in fresh water. The hands of the operator, the dishes and pipettes used, were carefully sterilized in the same way. For the same reason, sea-water was used which had been raised to a temperature of 60° C., cooled and aërated. After washing in fresh water, the *Amphitrite* was placed in a dish of sterilized sea-water until the eggs were removed. In the following four experiments the eggs were removed from the female at 2.10 P. M. July 30, and were at once transferred very carefully to fresh sterilized sea-water.

Experiment 1.—The object of this experiment was to test the effect of transferring from one dish to another. In order to get a standard amount of agitation, the eggs were allowed to fall, one drop at a time, from the mouth of a pipette held one inch above the water. The different lots of eggs and the time each was transferred are given below:

1	control, transferred	2:10 P. M.
2	transferred	2:27 P. M.
3	"	2:43 P. M.
4	"	2:58 P. M.
5	"	3:13 P. M.
6	"	3:43 P. M.
7	"	4:13 P. M.
8	"	4:43 P. M.

The dishes containing the transferred eggs were left undisturbed until 10 P. M., when eggs were taken from 1, 2, 4, 6, 8

and examined. Care was taken to avoid disturbing those left in the dishes.

The control showed nearly all eggs unchanged; in a few the germinal vesicle had broken down and they were darker (more opaque) in color; a few had started to segment.

Lot 2. The germinal vesicle had broken down in nearly all; a "perivitelline space" found in about 20 per cent., but was rather small in most of this number. Most of the eggs were light (translucent) in color.

Lot 4. An irregular "perivitelline space" in 40–50 per cent. The germinal vesicle was broken down in practically all, the light-colored as well as the dark. There were a few extra-ovates.

Lot 6. The germinal vesicle broken down in nearly all; a "perivitelline space" in 40–50 per cent., irregular in some; a smaller number are blackened.

Lot 8. A prominent "perivitelline space" and contracted protoplasm in 50–60 per cent.; the rest have the germinal vesicle intact.

Amphitrite eggs frequently begin differentiation if left in seawater entirely undisturbed. This is shown in the above control. The experiment so far disclosed no marked phenomena, and I give the above descriptions to indicate the comparatively uniform development at this time. No evidence of normal segmentation was found at any time in this and the three following experiments.

All the dishes were again examined at 9:30 the next morning, as the advanced stages afford a better means of testing the effects of transference. Below is given the estimated number of ciliated eggs found in 2,000 of each lot. Aside from the swimming eggs, the different lots were in practically the same condition as on the previous evening. No further description is then necessary.

Lot Number.	Time Transferred from Beginning.	Number Ciliated in 2,000 Eggs.
1	0 min.	0
2	17 "	0
3	33 "	10
4	48 "	10
5	63 "	4
6	93 "	60
7	123 "	10
8	153 "	10

Experiment 2. — The object of the experiment was to test the effect of a more violent method of transferring. The eggs were taken up in a pipette and squirted with moderate pressure into the dish of sterilized sea-water from a distance of two or three inches; then water in the dish was taken up three times and squirted at the surface. The control was simply transferred.

An examination of these eggs was made at 10:15 P. M., when their condition was not much different from those in Experiment 1, except that more showed effects of the agitation. The next morning, 10:15 A. M., the following results were obtained:

Lot Number.	Time Transferred from Beginning.	Number Ciliated in 2,000 Eggs.
1 control.	0 min.	0
2	7 "	2
3	23 "	10
4	38 "	40
5	55 "	0
6	70 "	3
7	100 "	20
8	130 "	4
9	160 "	0

Experiment 3. — Eggs were transferred in the following lots and squirted moderately as in Experiment 2. Thereafter they were squirted again moderately at frequent (10–15 min.) intervals, up to 4:40 P. M. Examined at 9 A. M. July 31.

Lot Number.	Time Transferred from Beginning.	Number Ciliated in 2,000 Eggs.
1 control.	0 min.	0
2	32 "	60
3	60 "	0
4	120 "	4

Experiment 4. — The eggs were squirted violently, the water vigorously agitated by squirting with a pipette, and then left undisturbed.

Condition at 8:45 A. M., July 31. Many fragments present.

Lot Number.	Time Transferred from Beginning.	Number Ciliated in 2,000 Eggs.
1 control.	0 min.	0
2	33 "	40
3	60 "	2
4	120 "	0

I have taken the above experiments as typical examples. I have occasionally obtained a much larger per cent. of swimming eggs, frequently a smaller number, and sometimes none. Accepting the number of swimming structures as a fair test of development of this kind, we may make again the following statements :

1. In the differentiation of unfertilized eggs of *Amphitrite*, produced by transference, squirting or other methods of agitation, there are at least two periods in which they are highly susceptible, one thirty to forty-five minutes, the other eighty to one hundred minutes after being put into sea-water.

I have attempted to depict this idea on ordinate paper, shown in the accompanying figures. Abscissas represent time from the beginning of an experiment, ordinates the relative number of

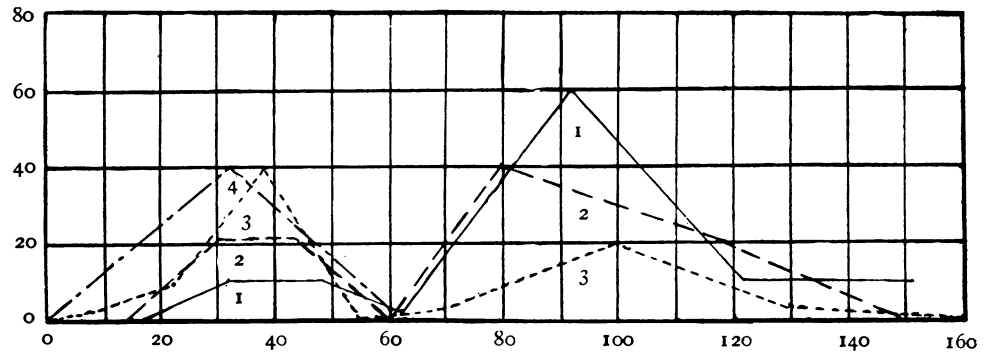

FIG. 1. 1. Gently transferred, experiment 1. 2. Very moderately squirted, experiment 26. 3. Moderately squirted, experiment 2. 4. Violently squirted, experiment 4.

swimming eggs in 2,000 of each lot. Fig. 1 gives the results of four experiments produced by different degrees of agitation. Fig. 2 shows all the observations of these four experiments combined in a single line ; where two observations were made at the same time their average is taken (in one case only). The dotted line gives my idea of the curve of susceptibility, as brought about by a moderate degree of shaking.

2. By comparing experiments 1, 2 and 3 (Figs. 1, 1, 2, 3) we find slight agitation is more effective in the second period than in the first ; rougher treatment causes more to develop in the first period, but injures some in the second.

3. Frequent and moderate squirting after thirty to forty-five minutes seems more effective than one hard squirting, after the same time. Compare experiments 3 and 4.

A comparison with fertilized eggs is of interest. The normal egg throws off the first polar body in less than thirty minutes after fertilization, and the first cleavage appears about thirty minutes later. According to Loeb's[1] view, the sperm in the case of parthenogenetic eggs acts simply to hasten, or accelerate, processes which are already present in the egg. It has frequently been noticed that the unfertilized egg of *Amphitrite*, if left undisturbed in sea-water, will often show some phenomena of differentiation. Assuming Loeb's theory as a working hypothesis, we should expect artificial means to be slower than fertilization. This proves to be the case; fertilized eggs develop cilia sooner

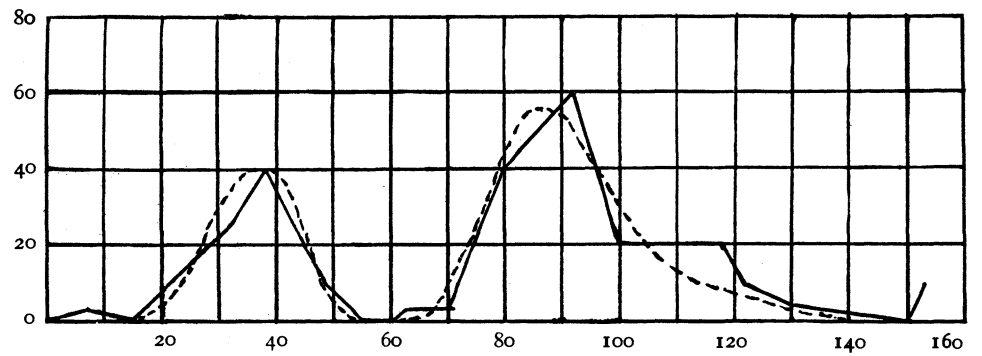

FIG. 2.

than those squirted. Sometimes there is not much difference in *Amphitrite*. Presumably, then the two critical periods mentioned correspond to processes in the normal egg that are active about the time for the appearance of the first polar body and the first cleavage; there is the same relative time between them. Further work is needed to prove this. Delage[2] states that the starfish egg is highly susceptible to "artificial fertilization" between the breaking down of the germinal vesicle and the appearance of the first polar body.

However this may be, it is certain that *there are processes of differentiation going on in the unfertilized eggs of Amphitrite which*

[1] Loeb, J., *Am. Jour. of Phys.*, 1901, Vol. IV., No. IX.

[2] Delage, Y., *Archiv d. Zool. Exper. et. Gen.*, 1901, T. IX., Nos. 2–3.

may be started into activity at definite intervals by mechanical agitation. These processes are, for the most part at least, independent of the processes that cause segmentation. I have noticed, as a rule, that the riper the eggs are the more cleavage is found, but I am convinced that it is never normal beyond the first few segmentations, if at all. It would seem, then, that the sperm introduces the active cause of this process.

It has been shown by Lyon[1] in the fertilized *Arbacia* egg, that there are recurring periods of susceptibility to KNC poisoning, and to lack of oxygen. Each period of susceptibility is followed by a period of resistance. On the other hand, in the unfertilized eggs of *Amphitrite*, there are at certain times unstable conditions, during which a small amount of agitation will set these unstable forces free, and lead to some definite characteristics of more advanced development (*i. e.*, production of cilia, etc.).

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[1] Lyon, E. P., *Am. Jour. of Phys.*, 1902, Vol. VII., No. 1.